DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

P95 P94/6018

# Memorandum

Date NOV 29 1995

From Director, Office of Device Evaluation (HFZ-400)
Center for Devices and Radiological Health (CDRH)

Subject Premarket Approval of BARD Diagnostic Sciences, Incorporated's BARD® BTA® Test - Action

To The Director, CDRH
ORA _____

ISSUE. Publication of a notice announcing approval of the subject PMA.

FACTS. Tab A contains a FEDERAL REGISTER notice announcing:

(1) a premarket approval order for the above referenced medical device (Tab B); and

(2) the availability of a summary of safety and effectiveness data for the device (Tab C).

RECOMMENDATION. I recommend that the notice be signed and published.

Susan Alpert, Ph.D., M.D.

Attachments
Tab A - Notice
Tab B - Order
Tab C - S & E Summary

DECISION.

Approved _____ Disapproved _____ Date _____

Prepared by Peter E. Maxim, CDRH, HFZ-440, 10/20/95, 594-1293

DEPARTMENT OF HEALTH AND HUMAN SERVICES

FOOD AND DRUG ADMINISTRATION

[DOCKET NO. _____]

BARD Diagnostic Sciences, Inc.; Premarket Approval of BARD® BTA® Test.

AGENCY: Food and Drug Administration, HHS.

ACTION: Notice.

SUMMARY: The Food and Drug Administration (FDA) is announcing its approval of the application by BARD Diagnostic Sciences, Inc., Redmond, WA, for premarket approval, under section 515 of the Federal Food, Drug, and Cosmetic Act (the act), of BARD® BTA® Test. After reviewing the recommendation of the Immunology Devices Panel, FDA's Center for Devices and Radiological Health (CDRH) notified the applicant, by letter on November 29, 1995, of the approval of the application.

DATE: Petitions for administrative review by (insert date 30 days after date of publication in the FEDERAL REGISTER).

ADDRESS: Written requests for copies of the summary of safety and effectiveness data and petitions for administrative review to the Dockets Management Branch (HFA-305), Food and Drug Administration, Rm. 1-23, 12420 Parklawn Drive, Rockville, MD 20857.

FOR FURTHER INFORMATION CONTACT:

Peter E. Maxim, Ph.D.

Center for Devices and Radiological Health (HFZ-440)

Food and Drug Administration

2098 Gaither Road

Rockville, MD 20850

301-594-1293.

SUPPLEMENTARY INFORMATION: On June 6, 1994, BARD Diagnostic Sciences, Inc., Redmond, WA, 98052, submitted to CDRH an application for premarket approval of BARD® BTA® Test. The BARD® BTA® rapid latex agglutination test is an in vitro device intended for the qualitative measurement of Bladder Tumor Associated Analytes in human urine to aid in the management of bladder cancer patients.

On September 21, 1995, the Immunology Devices Panel, an FDA advisory panel, reviewed and recommended approval of the application.

On November 29, 1995, CDRH approved the application by a letter to the applicant from the Director of the Office of Device Evaluation, CDRH.

A summary of the safety and effectiveness data on which CDRH based its approval is on file in the Dockets Management Branch (address above) and is available from that office upon written request. Requests should be identified with the name of the device and the docket number found in brackets in the heading of this document.

## OPPORTUNITY FOR ADMINISTRATIVE REVIEW

Section 515(d)(3) of the act (21 U.S.C. 360e(d)(3)) authorizes any interested person to petition, under section 515(g) of the act (21 U.S.C. 360e(g)), for administrative review of CDRH's decision to approve this application. A petitioner may request either a formal hearing under part 12 (21 CFR part 12) of FDA's administrative practices and regulations or a review of the application and CDRH's action by an independent advisory committee of experts. A petition is to be in the form of a petition for reconsideration under 10.33(b) (21 CFR 10.33(b)). A petitioner shall identify the form of review requested (hearing or independent advisory committee) and shall submit with the petition supporting data and information showing that there is a genuine and substantial issue of material fact for resolution through administrative review. After reviewing the petition, FDA will decide whether to grant or deny the petition and will publish a notice of its decision in the FEDERAL REGISTER. If FDA grants the petition, the notice will state the issue to be reviewed, the form of the review to be used, the persons who may participate in the review, the time and place where the review will occur, and other details.

Petitioners may, at any time on or before (insert date 30 days after date of publication in the FEDERAL REGISTER), file with the Dockets Management Branch (address above) two copies of each petition and supporting data and information, identified with the name of the device and the docket number found in brackets in the heading of this document. Received petitions may be seen in the office above between 9 a.m. and 4 p.m., Monday through Friday.

This notice is issued under the Federal Food, Drug, and Cosmetic Act (secs. 515(d), 520(h), (21 U.S.C. 360e(d), 360j(h)) and under authority delegated to the Commissioner of Food and Drugs (21 CFR 5.10) and redelegated to the Director, Center for Devices and Radiological Health (21 CFR 5.53).

Dated:_____.

_____

DEPARTMENT OF HEALTH & HUMAN SERVICES — Public Health Service

Food and Drug Administration
2098 Gaither Road
Rockville MD 20850

Mr. Glen Paul Freiberg, RAC
Vice President, Operations
Bard Diagnostic Sciences, Inc.
12277 134th Ct. N.E., #100
Redmond, Washington 98052

NOV 29 1995

Re: P940018
Bard® BTA® Test Kit
Filed: June 6, 1994
Amended: July 13; September 7; and December 12, 1994;
March 24; June 27; September 1; and October 30, 1995.

Dear Mr. Freiberg:

The Center for Devices and Radiological Health (CDRH) of the Food and Drug Administration (FDA) has completed its review of your premarket approval application (PMA) for the Bard® BTA® Test Kit. This rapid latex agglutination test is an in vitro device intended for the qualitative measurement of Bladder Tumor Associated Analytes in human urine to aid in the management of bladder cancer patients. We are pleased to inform you that the PMA is approved subject to the conditions described below and in the "Conditions of Approval" (enclosed). You may begin commercial distribution of the device upon receipt of this letter.

CDRH will publish a notice of its decision to approve your PMA in the FEDERAL REGISTER. The notice will state that a summary of the safety and effectiveness data upon which the approval is based is available to the public upon request. Within 30 days of publication of the notice of approval in the FEDERAL REGISTER, any interested person may seek review of this decision by requesting an opportunity for administrative review, either through a hearing or review by an independent advisory committee, under section 515(g) of the Federal Food, Drug, and Cosmetic Act (the act).

Failure to comply with the conditions of approval invalidates this approval order. Commercial distribution of a device that is not in compliance with these conditions is a violation of the act.

You are reminded that as soon as possible, and before commercial distribution of your device, that you must submit an amendment to this PMA submission with copies of all approved labeling in final printed form.

All required documents should be submitted in triplicate, unless otherwise specified, to the address below and should reference the above PMA number to facilitate processing.

PMA Document Mail Center (HFZ-401)
Center for Devices and Radiological Health
Food and Drug Administration
9200 Corporate Blvd.
Rockville, Maryland 20850

If you have any questions concerning this approval order, please contact Peter E. Maxim, Ph.D. at (301) 594-1293.

Sincerely yours,

Susan Alpert, Ph.D., M.D.
Director
Office of Device Evaluation
Center for Devices and
Radiological Health

Enclosure

## CONDITIONS OF APPROVAL

APPROVED LABELING. As soon as possible, and before commercial distribution of your device, submit three copies of an amendment to this PMA submission with copies of all approved labeling in final printed form to the PMA Document Mail Center (HFZ-401), Center for Devices and Radiological Health, Food and Drug Administration (FDA), 9200 Corporate Blvd., Rockville, Maryland 20850.

ADVERTISEMENT. No advertisement or other descriptive printed material issued by the applicant or private label distributor with respect to this device shall recommend or imply that the device may be used for any use that is not included in the FDA approved labeling for the device. If the FDA approval order has restricted the sale, distribution and use of the device to prescription use in accordance with 21 CFR 801.109 and specified that this restriction is being imposed in accordance with the provisions of section 520(e) of the act under the authority of section 515(d)(1)(B)(ii) of the act, all advertisements and other descriptive printed material issued by the applicant or distributor with respect to the device shall include a brief statement of the intended uses of the device and relevant warnings, precautions, side effects and contraindications.

PREMARKET APPROVAL APPLICATION (PMA) SUPPLEMENT. Before making any change affecting the safety or effectiveness of the device, submit a PMA supplement for review and approval by FDA unless the change is of a type for which a "Special PMA Supplement-Changes Being Effected" is permitted under 21 CFR 814.39(d) or an alternate submission is permitted in accordance with 21 CFR 814.39(e). A PMA supplement or alternate submission shall comply with applicable requirements under 21 CFR 814.39 of the final rule for Premarket Approval of Medical Devices.

All situations which require a PMA supplement cannot be briefly summarized, please consult the PMA regulation for further guidance. The guidance provided below is only for several key instances.

A PMA supplement must be submitted when unanticipated adverse effects, increases in the incidence of anticipated adverse effects, or device failures necessitate a labeling, manufacturing, or device modification.

A PMA supplement must be submitted if the device is to be modified and the modified device should be subjected to animal or laboratory or clinical testing designed to determine if the modified device remains safe and effective.

A "Special PMA Supplement - Changes Being Effected" is limited to the labeling, quality control and manufacturing process changes specified under 21 CFR 814.39(d)(2). It allows for the **addition** of, but **not the replacement** of previously approved, quality control specifications and test methods. These changes may be implemented before FDA approval upon acknowledgment by FDA that the submission is being processed as a "Special PMA Supplement - Changes Being Effected." This acknowledgment is in addition to that issued by the PMA Document Mail Center for all PMA supplements submitted. **This procedure is not applicable to changes in device design, composition, specifications, circuitry, software or energy source.**

Alternate submissions permitted under 21 CFR 814.39(e) apply to changes that otherwise require approval of a PMA supplement before implementation of the change and include the use of a 30-day PMA supplement or annual postapproval report. FDA must have previously indicated in an advisory opinion to the affected industry or in correspondence with the applicant that the alternate submission is permitted for the change. Before such can occur, FDA and the PMA applicant**(s)** involved must agree upon any needed testing protocol, test results, reporting format, information to be reported, and the alternate submission to be used.

POSTAPPROVAL REPORTS. Continued approval of this PMA is contingent upon the submission of postapproval reports required under 21 CFR 814.84 at intervals of 1 year from the date of approval of the original PMA. Postapproval reports for supplements approved under the original PMA, if applicable, are to be included in the next and subsequent annual reports for the original PMA unless specified otherwise in the approval order for the PMA supplement. Two copies identified as "Annual Report" and bearing the applicable PMA reference number are to be submitted to the PMA Document Mail Center (HFZ-401), Center for Devices and Radiological Health, Food and Drug Administration, 9200 Corporate Blvd., Rockville, Maryland 20850. The postapproval report shall indicate the beginning and ending date of the period covered by the report and shall include the following information required by 21 CFR 814.84:

(1) Identification of changes described in 21 CFR 814.39(a) and changes required to be reported to FDA under 21 CFR 814.39(b).

(2) Bibliography and summary of the following information not previously submitted as part of the PMA and that is known to or reasonably should be known to the applicant:

(a) unpublished reports of data from any clinical investigations or nonclinical laboratory studies involving the device or related devices ("related" devices include devices which are the same or substantially similar to the applicant's device); and

(b) reports in the scientific literature concerning the device.

If, after reviewing the bibliography and summary, FDA concludes that agency review of one or more of the above reports is required, the applicant shall submit two copies of each identified report when so notified by FDA.

ADVERSE REACTION AND DEVICE DEFECT REPORTING. As provided by 21 CFR 814.82(a)(9), FDA has determined that in order to provide continued reasonable assurance of the safety and effectiveness of the device, the applicant shall submit 3 copies of a written report identified, as applicable, as an "Adverse Reaction Report" or "Device Defect Report" to the PMA Document Mail Center (HFZ-401), Center for Devices and Radiological Health, Food and Drug Administration, 9200 Corporate Blvd., Rockville, Maryland 20850 within 10 days after the applicant receives or has knowledge of information concerning:

(1) A mixup of the device or its labeling with another article.

(2) Any adverse reaction, side effect, injury, toxicity, or sensitivity reaction that is attributable to the device and

(a) has not been addressed by the device's labeling or

(b) has been addressed by the device's labeling, but is occurring with unexpected severity or frequency.

(3) Any significant chemical, physical or other change or deterioration in the device or any failure of the device to meet the specifications established in the approved PMA that could not cause or contribute to death or serious injury but are not correctable by adjustments or other maintenance procedures described in the approved labeling. The report shall include a discussion of the applicant's assessment of the change, deterioration or failure and any proposed or implemented corrective action by the applicant. When such events are correctable by adjustments or other maintenance procedures described in the approved labeling, all such events known to the applicant shall be included in the Annual Report described under "Postapproval Reports" above unless specified otherwise in the conditions of approval to this PMA. This postapproval report shall appropriately categorize these events and include the number of reported and otherwise known instances of each category during the reporting period. Additional information regarding the events discussed above shall be submitted by the applicant when determined by FDA to be necessary to provide continued reasonable assurance of the safety and effectiveness of the device for its intended use.

REPORTING UNDER THE MEDICAL DEVICE REPORTING (MDR) REGULATION. The Medical Device Reporting (MDR) Regulation became effective on December 13, 1984, and requires that all manufacturers and importers of medical devices, including in vitro diagnostic devices, report to FDA whenever they receive or otherwise became aware of information that reasonably suggests that one of its marketed devices

(1) may have caused or contributed to a death or serious injury or

(2) has malfunctioned and that the device or any other device marketed by the manufacturer or importer would be likely to cause or contribute to a death or serious injury if the malfunction were to recur.

The same events subject to reporting under the MDR Regulation may also be subject to the above "Adverse Reaction and Device Defect Reporting" requirements in the "Conditions of Approval" for this PMA. FDA has determined that such duplicative reporting is unnecessary. Whenever an event involving a device is subject to reporting under both the MDR Regulation and the "Conditions of Approval" for this PMA, you shall submit the appropriate reports required by the MDR Regulation and identified with the PMA reference number to the following office:

Division of Surveillance Systems (HFZ-531)
Center for Devices and Radiological Health
Food and Drug Administration
1350 Piccard Drive, Room 240
Rockville, Maryland 20850
Telephone (301) 594-2735

Events included in periodic reports to the PMA that have also been reported under the MDR Regulation must be so identified in the periodic report to the PMA to prevent duplicative entry into FDA information systems.

Copies of the MDR Regulation and an FDA publication entitled, "An Overview of the Medical Device Reporting Regulation," are available by written request to the address below or by telephoning 1-800-638-2041.

Division of Small Manufacturers Assistance (HFZ-220)
Center for Devices and Radiological Health
Food and Drug Administration
5600 Fishers Lane
Rockville, Maryland 20857

**SUMMARY OF SAFETY AND EFFECTIVENESS**

I. **General Information**

Device Generic Name: Latex agglutination test for measuring bladder tumor associated analytes in urine.

Device Trade Name: Bard® BTA® Test

Applicant's name and address:

Bard® Diagnostic Sciences, Inc., Subsidiary of C.R. Bard®, Inc.
12277 134th Court NE
Redmond, Washington, 98052

Premarket Approval Application (PMA) Number: P940018

Date of Panel Recommendation: September 21, 1995

Date of notice of approval to applicant: November 29, 1995

## II. Indications for Use

The Bard® BTA® rapid latex agglutination test is an *in vitro* device intended for the qualitative measurement of Bladder Tumor Associated Analytes in human urine to aid in the management of bladder cancer patients.

### Background

#### Bladder Cancer

Bladder cancer is the fourth most common form of cancer in men and the eighth most common form in women in the United States.[1] Approximately 75 to 85 per cent of the patients present with transitional cell carcinoma (TCC) confined to the superficial mucosa of the bladder.[2] The risk of recurrence in these patients is 75 per cent[2]. Since bladder cancer has a greater incidence of recurrence during the first two years after the diagnosis of primary cancer[3], persons with a prior diagnosis of bladder cancer receive a cystoscopic examination every three months for the first two years, every six months for the second two years, and annually thereafter, to monitor for recurrence of the cancer. Patients may also be monitored for recurrence of bladder cancer by urine cytology.

Urine cytology as a monitoring tool has a variable sensitivity depending on the skill of the cytologist and tumor stage and grade. Lowest sensitivity is reported for early stage disease.[4,5]

Bladder Tumor Associated Analytes

Bladder Tumor Associated Analytes (BTA) are found in the urine of 40 per cent of bladder cancer patients. They have been shown to contain high molecular weight proteolytic degradation polypeptides of molecular weight 16 to 165 kD. Immunologically they appear to consist of complexes of basement membrane proteins and in some cases may also contain immunoglobulin G.

Three mechanisms have been postulated for the appearance of these bladder tumor analytes in urine of some bladder cancer patients: (1) tumor invasion of the basement membrane; (2) production by the tumor itself; and (3) a combination of these which may be linked with the body's immune response.[6-14]

## III. Device Description

The BTA® test combines the principles of latex agglutination and paper chromatography for the qualitative detection of BTA in urine. The latex reagent contains a blue colloidal dye and a yellow water soluble dye that results in an overall green color to the latex reagent. If agglutination has occurred, the blue colloidal dye is trapped in the test well by the agglutinated latex particles. The yellow color of the water-soluble dye diffuses up the paper strip signaling a positive result. If agglutination has not occurred, the blue colloidal dye diffuses up the test strip along with the yellow dye resulting in a green color. Positive and negative controls are supplied along with a color guide for test interpretation.

## IV. Alternative Procedures and Practices

Cystoscopy of the bladder, is the diagnostic standard of care for monitoring patients with a history of bladder cancer, since visualization is always necessary. This method is an interventional procedure, limited to detection of those tumors that can be visualized. Cystoscopic examination may lead to either a directed or blind biopsy of the bladder in those patients with suspicious findings.

Urine cytology is a tool with low and variable sensitivity depending on the skill of the cytologist and tumor stage and grade[4]. Lowest sensitivity is reported for early stage disease.[5,6]

A urinalysis test for blood in urine may be indicative of cancer as well as other urological conditions. An intravenous pyelogram also can be used to look for cancer in the upper urinary tract.

## V. Marketing History

The Bard® BTA® Test Kit is currently marketed in the following countries: Albania, Australia, Austria, Bahrain, Bangladesh, Belgium, Bolivia, Bulgaria, Canada, Chile, CIS, Costa Rica, Croatia, Cyprus, Czech Republic, Denmark, Ecuador, Egypt, Estonia, Finland, France, Germany, Hong Kong, Hungary, Iceland, India, Ireland, Israel, Italy, Kenya, Kuwait, Latvia, Lebanon, Lithuania, Malaysia, Malta, Mauritius, Nepal, Netherlands, New Zealand, Norway, Pakistan, Paraguay, Peru, Philippines, Poland, Portugal, Qatar, Romania, Saudi Arabia, Singapore, Slovak Republic, Slovenia, South Africa, spain, Sri Lanka, Sweden, Switzerland, Syria, Taiwan, Turkey, United arab Emerits,

United Kingdom, Uruguay, Venezuela, Wales, Yemen. The BTA test has not been recalled or withdrawn from the market for any reason in any country.

## VI. Adverse Effects of the Device on Health

A false positive BTA® result may lead the urologist to perform more intensive and invasive diagnostic procedures. Such procedures might include random blind, or cold cup biopsies under general or spinal anesthesia, or intravenous pyelograms.

False negative BTA® results may lead the physician and patient to have a false sense of security that no lesions will be seen during cystoscopy. Thus, full attention to the search for lesions may not be given.

### Contraindications

- Do not use kit components after expiration date.
- Do not use kit components from other lots of this test.
- Do not reuse disposable test stations, test strips, droppers, microtubes, or pipette tips. Discard after single use.
- Do not touch the pad portion of the test strip or use test strips that are damaged.
- Do not use test kits that are delivered damaged or that show leakage from reagent vials.

### Warnings

- For in vitro diagnostic use.
- Buffer, Controls and Reagent each contain 0.1% sodium azide (NaN3) which, if allowed to accumulate, can form

explosive compounds in lead and/or copper plumbing. When disposing of Buffer, Controls, or Reagent, flush disposal area with large volumes of water to prevent possible formation of such explosive compounds.

- Human source material used for the preparation of the BTA Reagent was tested and found to be negative for antibodies to Human Immunodeficiency Virus Type 1 (HIV-1), for Hepatitis B Surface Antigen (HBs Ag) and for nucleic acid sequences characteristic of Human Immunodeficiency Virus Type 2 (HIV-2) and Hepatitis C Virus (HCV). Human source material used for the preparation of the Positive Control was heat/low pH inactivated. No available test can offer absolute assurance of the absence of infectious agents. Handle these reagents and all materials coming into contact with them as potentially infectious.

**Precautions**

- To avoid cross-contamination of samples, use a new dropper, tube, and pipette tip for each urine sample.
- Wear disposable gloves. Wash hands thoroughly after handling specimens and kit contents.

**Specimen collection, storage, preparation precautions**

- Do not use paper or foam cups for urine specimen collection or storage.
- Do not test urine specimens that have been heated or frozen.
- Do not use samples from timed urine collections (24 hour urines).
- Avoid testing urine with elevated levels of leukocytes in the urine (positive urinalysis test strip reading).

- The effects of radiation therapy within three months or systemic chemotherapy within 30 days on the BTA test is unknown. Testing prior to these time frames is not recommended.
- BTA testing should not be performed in patients until at least 30 days after intravesical therapy. The following intravesical therapies were evaluated: BCG, mitomycin C, Thiotepa, bropiramine (investigational) and interferon (investigational) and interferon (investigational). Patients receiving intravesical agents other than those evaluated should be tested (after 30 days) at the discretion of the physician.
- The effects of experimental drugs on the BTA test are unknown. Patients taking an experimental or investigational drug should not be tested until the drug has been fully excreted.
- Semen is known to cause false positive BTA test results. First voided urine following ejaculation should not be tested. This is a particular problem after some cases of transurethral resection of the prostate where retrograde ejaculation may occur.
- For trauma to the bladder or urinary tract due to surgery, biopsy, etc., the physician should allow ample time for trauma recovery before using the test.

**Limitations**

- Results of the BTA test should not be interpreted as absolute evidence for the presence or absence of TCC of the bladder. Elevated levels may be observed in urine from patients with recent surgery, biopsy or other invasive trauma to the bladder or urinary tract.
- Active infections of the genitourinary tract, renal or bladder calculi, and positive leukocyte reading on

urinalysis test strip may cause false positive test results.

- TCC of the kidney or ureters may give a positive BTA test result.
- The result from the BTA test should be used only in conjunction with information available from the clinical evaluation of the patient and other diagnostic procedures.
- The BTA test should not be used as a screening test.

## VII. SUMMARY OF STUDIES

### A. Preclinical Studies

#### 1. Characterization of the Positive Reactivity of the Test.

The applicant has shown by molecular sieve chromatography that the test reacts with high molecular weight proteolytic degradation polypeptides of molecular weight 16 to 165 kD present in urine of 40 per cent of persons with TCC of the bladder. These analytes are absent from urine of most normal persons. The sponsor has shown also that the very same fractions of urine from a cancer patient react immunologically with antibodies to collagen IV and fibronectin.

The applicant has determined also that the BTA® test does agglutinate with the following components of bladder basement membrane: collagen IV, laminin, and fibronectin, as well as immunoglobulin G (IgG), fibrinogen and fibrin degradation products.

2. **Analytical Sensitivity/Limits of Detection/Test Cutoff**

The analytical sensitivity of the BTA® Test (the cutoff between positive and negative results) was determined to be 9.8 micrograms/mL of urine by dilution analysis of a sample containing a high concentration of affinity purified BTA (2500 micrograms/mL). The analytical sensitivity of the test determined by standard curve analysis was 9-10 µg Type IV collagen/mL. Since BTA may have variable composition from patient to patient, human placental Type IV collagen was used as the surrogate standard and control material in the test and for in-house quality control.

The data indicated correlation by weight between pure Type IV collagen and affinity purified BTA. It was concluded, therefore, that it was acceptable to use Type IV collagen as a surrogate quality control and calibration material.

3. **Interference Studies**

Six common over-the-counter and prescription drugs (acetaminophen, acetyl sulfisoxazole, acetylsalicylic acid, ampicillin, chlorothiazide, and ciprofloxacin) 8 vitamins (retinol, thiamine, riboflavin, pyridoxine, cyanocobalamine, ascorbic acid, cholecalciferol, and tocopherol); 11 therapeutic agents (doxorubicin-HCl, mitomycin, nitrofurantoin, phenazopyridine - HCl, thiotepa, trimethoprin, BCG, hytrin, proscar, premarin, and flutamide); and 22 urine components (albumin, ammonium chloride, bilirubin, C-Reactive protein, calcium chloride, sodium chloride, creatinine, glucose,

haptoglobin, hemoglobin, immunoglobulins G, A, E, and M, methylene blue, beta-2-microglobulin, potassium chloride, sodium sulfate, transferrin, urea, and uric acid); red and white blood cells, and specific gravity were tested to determine if there was positive or negative interference in conducting the assay.

It was shown that most drugs, vitamins and urine components at the levels tested did not interfere with the test. Exceptions were ascorbic acid (vitamin C) at 25 mg/mL, phenazopyridine-HCl at 1 mg/mL, IgG at greater than 31.25 µg/mL of urine, and white blood cells in excess of approximately $10^4$/mL of urine.

4. **Sample Dilution Study**

Ten BTA® positive urine samples were serially diluted to establish the effect of sample dilution on the sensitivity of the test. The studies indicated that the assay had greatest sensitivity when the test was conducted with undiluted urine. For this reason, the user is instructed to run the test on undiluted urine specimens.

5. **Sample Centrifugation Study**

A study was conducted to determine if cellular debris or other particulate matter interfered with the assay performance. It was concluded that they did not and, therefore, samples do not need to be centrifuged before using the assay.

6. **Reproducibility Studies (within day, between day, lot-to-lot, and between laboratory variation)**

Reproducibility studies were performed on 3 lots of BTA® latex reagents to determine within day, between day, lot-to-lot, and between laboratory variation. These were conducted at six laboratories on 10 replicates of 14 different samples containing varying levels of BTA over 5 days. Qualitative reproducibility was near total agreement except with samples near the positive/negative cutoff which is to be expected for qualitative tests.

7. **High-Dose Hook Effect (Prozone) Study**

Patient samples with high levels of test analyte can cause a paradoxical decrease in the ability to detect test analyte in agglutination assays. This phenomenon is known as prozone interference or high-dose hook effect. With the assay, a sample containing 4000 micrograms/mL Type IV collagen was tested and remained positive. This demonstrated that levels of analyte up to 400 times that at the test cutoff were not associated with a high-dose-hook-effect, and samples containing high levels of BTA should give a true positive result.

8. **Stability Studies**

Tests were completed to determine sample stability, kit expiration dating, and kit shipping conditions.

(a) **Sample Stability**

Accelerated and real-time testing were completed on six human urines (4 positive, one border-line and one negative) using one lot of BTA® test. These urines were tested at 37°C, ambient temperature, and at 2-8°C over 1-17 days. Several freeze thaw cycles were also tested.

These studies showed that samples had variable stability. Repeat testing was reproducible up to 48 hours after urine collection, if held refrigerated. Frozen samples maintained reactivity if frozen within 24 hours of collection. However, to ensure accurate results, freezing is not recommended for routine practice.

(b) **Kit Stability**

The stability of the BTA® test was determined by assaying in duplicate Type IV collagen standards at 2, 5, 10, 12, 15, and 200 micrograms/mL, the set's own positive and negative controls, and 3 positive and 3 negative patient urine samples with four different manufactured lots. The lots were tested at 2-8°C, real time; heat stressed for two 18-hour cycles of 50°C, and then at 2-8°C for the remainder of the study; freeze/thaw stressed (two 24-hour freeze/thaw cycles) and then held at 2-8°C for the remainder of the study; and accelerated testing at 30°C and

45°C until the test failed to reproduce the expected result. The kits were also subjected to thermal toleration tests at 60°C, 70°C, and 80°C for 2, 6, and 12 hours.

Real-time studies indicated a kit shelf life of 24 months for all reagents when stored at 2 - 8°C.

(c) **Shipping Conditions**

1) Shipping conditions were determined by assaying four manufactured lots that were subjected to the following various stress conditions. They were heat stressed for two 18-hour cycles of 50°C and then held at 2-8°C for the duration of the study. They were subjected to two sets of twenty-four hour freeze/thaw conditions. The protocol for the freeze/thaw conditions consisted of -5°C for 6 hours, then -25°C for 18 hours, then 24 hours at 2 -8°C. Then the entire freeze cycle was repeated. The kits were then held at 2-8°C for the remainder of the study. Accelerated studies were performed at 30°C and 45°C until reagent failure. The kits were subjected also to thermal toleration tests at 60°C, 70°C, and 80°C for 2, 6, and 12 hours.

2) Three different manufactured lots were subjected to the same stress conditions

as above, but after 16 to 18 months of shelf life (as opposed to 3 to 5 months).

3) One manufactured lot was subjected to a high-temperature toleration study to determine the maximum temperature reagents will tolerate, as well as real-time stability testing stressed at 60°C over 92 cycles of 18 hours each.

The BTA® Test was shown to tolerate a wide range of shipping temperatures. The test continued to perform to specifications for 24 months after undergoing two 24-hour freeze/thaw cycles and for 24 months after being subjected to extremes of temperature.

## B. Clinical Studies

Sixteen investigational sites participated in two clinical studies. Two sites participated in the Specificity Trial 9301 and fourteen sites in the Sensitivity Monitoring Trial 9201. Longitudinal patient studies in the second trial, demonstrated the clinical utility of the Bard® BTA® Test.

### 1. Specificity Trial 9301

#### A. Objective

Specificity Trial 9301 was a cross-sectional study to determine the specificity and expected results for the test in populations of healthy volunteers and patients with a variety of urological and

nonurological illnesses without prior diagnosis or suspicion of bladder cancer.

B. **Investigators**

The Specificity Trial 9301 was conducted at 2 different geographical sites. See TABLE 1.

**TABLE 1 Specificity trial (9301) Investigator**

| | NAME AND ADDRESS OF INVESTIGATOR | SUBJECTS ENROLLED | SAMPLES ENROLLED |
|---|---|---|---|
| 1. | Kim Riddell, M.D.<br>Ernest Kawamoto, M.D.<br>Cooperative Medical<br>Laboratory Providence<br>Hospital and<br>Clinics<br>Everett, WA | 465 | 478 |
| 2. | George Adams, M.D.<br>SORRA N.C. Research Center<br>Birmingham, AL | 193 | 196 |
| | TOTALS | 658 | 674 |

C. **Populations Studied**

Urine samples from 658 volunteers without apparent disease and 11 different groups of patients without bladder cancer, representing 674 visits, were evaluated at 2 centers. All positive test results in the population were assumed to be false positives.

Inclusion criteria for Specificity Trial 9301 were subjects without apparent disease who were 35 years old or older and free of any malignancy or significant concurrent medical condition(s) or disease. Some healthy subjects were current smokers and some were non-smokers. No age requirements existed for patients with various conditions other than bladder cancer. The conditions included benign non-genitourinary disease; patients with urethritis, cystitis, prostatitis, and benign prostatic hyperplasia (BPH); patients with other genitourinary cancers (prostate, renal, ovarian, etc.); patients with non-genitourinary cancers (lung, breast, colon, lymphoma); patients with bladder trauma; and patients with symptomatic sexually-transmitted diseases.

Exclusion criteria for the specificity trial were:

- a history or clinical evidence of bladder cancer or any kind of metastases to the bladder,
- diagnosis or treatment for a malignancy within the last 12 months (except localized skin cancer and as defined above).
- trauma to the bladder within the last 14 days (e.g., indwelling catheterization, urinary tract surgery, cystoscopy) for all patients except those in the Bladder Trauma category,
- a medical condition(s) or disease in more than one study category (a patient with multiple disease entities is acceptable [e.g., diabetes and arthritis]),
- Pyridium (phenazopyridine hydrochloride) within three days prior to urine collection, or
- inability of patient to give consent.

A total of twelve subjects were eliminated from the database due to protocol violations. Nine were healthy subjects less than 35 years of age enrolled by mistake. One person's BTA® test was performed too long (48 hours) after sample collection. Another cancer patient was in remission and had no active cancer. One subject sample was performed on a bladder wash sample.

The 646 evaluable subjects had a mean age of 53 years, ranging from 15 to 96 years of age. These subjects included 211 apparently healthy individuals, 104 patients with non-genitourinary malignancies or diseases, and 331 patients with genitourinary malignancies, diseases, or trauma. There were 48 percent females and 52 percent males in all of the groups. In the 211 healthy individuals, there were 102 males and 109 females. The majority, 88 percent, were Caucasian. Of those remaining, 9 percent were Black, and 3 percent were of other racial origin. (See Table 2).

Table 2 **Specificity Trial 9301, Demographics of All Evaluable Subjects (N = 646)**

| | | ALL SITES (N = 646) |
|---|---|---|
| Age | *Mean* | 53 |
| | *Range* | 15-96 |
| | *< 50 years* | 310 (48%) |
| | *50-69 years* | 196 (30%) |
| | *≥ 70 years* | 140 (22%) |
| Gender | *Female* | 312 (48%) |
| | *Male* | 334 (52%) |
| Race | *Black* | 60 (9%) |
| | *Caucasian* | 567 (88%) |
| | *Other* | 19 (3%) |

Note: Protocol violation subjects have been excluded from this table.

Eleven categories of subjects were studied. The 331 genitourinary diseases and malignancies group included patients with benign prostatic hyperplasia (BPH) or cystitis (35) , symptomatic sexually transmitted diseases (STD) (65), benign renal diseases (36), and miscellaneous diseases (56), which included 23 urinary tract infections (UTI), prostatitis, incontinence, and other disorders. The malignancies evaluated were prostate carcinoma and renal cell carcinoma (44) and other nonbladder genitourinary malignancies (36). Fifty-nine subjects with recent genitourinary trauma were also tested. (See Table 3.)

Table 3. The following table shows specificity estimates for high and low specificity category groups.

| Pooled Across Sites | Leukocyte Status | Total | Spec. (%) |
|---|---|---|---|
| All High Specificity Categories | Negative | 370 | 96 |
| | Positive | 12 | 83 |
| Healthy Smoker (HS) | Negative | 82 | 95 |
| | Positive | 0 | |
| Healthy Nonsmoker (HNS) | Negative | 123 | 96 |
| | Positive | 3 | 100 |
| Benign Non-GU Disease (DCH) | Negative | 49 | 94 |
| | Positive | 1 | 100 |
| Non-GU Cancer (NGUC) | Negative | 49 | 100 |
| | Positive | 4 | 75 |
| BPH/Cystitis (BP/C) | Negative | 35 | 94 |
| | Positive | 0 | |
| Nonbladder GU Cancer (GUCA) | Negative | 32 | 94 |
| | Positive | 4 | 75 |
| All Low Specificity Categories | Negative | 205 | 89 |
| | Positive | 52 | 35 |
| Benign Renal Disease (BNRL) | Negative | 28 | 89 |
| | Positive | 7 | 71 |
| Misc. GU Disease (MSGU) | Negative | 47 | 94 |
| | Positive | 9 | 44 |
| Prostate/Renal Cancer (P/RC) | Negative | 40 | 95 |
| | Positive | 3 | 0 |
| Bladder Trauma (BLTR) | Negative | 40 | 75 |
| | Positive | 18 | 22 |
| Sexually Transmitted Disease (STD) | Negative | 50 | 90 |
| | Positive | 15 | 33 |

Only those cases with leukocyte results are included in the table above.

When subjects with leukocyte positive urine are removed, all categories met the protocol specificity criteria except Bladder Trauma. In subjects with negative or trace amounts of leukocytes in the urine, the BTA test correlates with the subjects' clinical evaluations.

Infection in the genitourinary tract, as indicated by a positive urinalysis leukocyte (granulocyte) strip test, may give a false positive BTA® result. Therefore, persons with a positive urinary leukocyte dip strip result were removed from each of the above categories except the "Genitourinary Trauma" group. All leukocyte urinalysis strip positive results were reported separately under the "Urine Positive for Leukocytes" group. (See Table 4.)

Table 4

BTA TEST SPECIFICITY RESULTS

| Patient Type | Number of Subjects | Test Negative (%) |
|---|---|---|
| History of Transitional Cell Carcinoma (No Evidence of Disease) | 348 | 80% |
| Healthy Subjects, Non-Genitourinary Diseases and Cancers (Urine negative for leukocytes) | 303 | 96% |
| Healthy Non-smokers | 123 | 96 |
| Healthy Smokers | 82 | 95 |
| Non-Genitourinary Diseases | 49 | 94 |
| Non-Genitourinary Cancers | 49 | 100 |
| Genitourinary Diseases and Cancers (Non-Bladder) (Urine negative for leukocytes) | 232 | 93% Total |
| Benign Prostatic Hyperplasia | 35 | 94 |
| Benign Renal Disease | 28 | 89 |
| Misc. Genitourinary Disease | 47 | 94 |
| Sexually Transmitted Diseases | 50 | 90 |
| Prostate/Renal Cancers | 40 | 95 |
| Other Genitourinary Cancers (Non-Bladder) | 32 | 94 |
| Genitourinary Trauma ≤ 14 Days | 59 | 59% |
| Leukocyte Negative[†] | 40 | 75 |
| Leukocyte Positive[†] | 18 | 22 |
| Leukocyte Unknown[†] | 1 | 100 |
| Urine Positive for Leukocytes (All patient types except Trauma category) | 46 | 52% |
| Urine Leukocyte[†] Status Unknown (All categories except Trauma) | 6 | 83% |
| TOTAL | 994 | |

† Leukocyte positive by urinalysis test strip

D. Results of Specificity Trial 9301

BTA® specificity was 96 percent in normal healthy persons (smokers and nonsmokers) and nongenitourinary diseases and malignancies. BTA® specificity was 93 percent in those with genitourinary diseases and malignancies. There were two categories containing a significant number of false positive results: those with leukocytes in the urine (48 percent) and those with genitourinary trauma less than 14 days before the test (41 percent). (See Table 4.)

Genitourinary trauma was defined as invasive trauma to the bladder or urinary tract due to surgery, biopsy, or intravesicular therapy. Catheterization was not considered to be genitourinary trauma.

2. Sensitivity Monitoring Trial 9201

A. Objective

Sensitivity Monitoring Trial 9201 was a multicenter, prospective, blinded study of patients who had a recent diagnosis or a history of bladder cancer. This study had two objectives:

(1) to determine the diagnostic sensitivity and specificity of the BTA® test compared to the reference method of biopsy confirmed cystoscopy performed at the same visit for determination of recurrence; and

(2) to compare BTA® diagnostic performance characteristics to those of VUC, to

demonstrate that the BTA® test was at least as sensitive as VUC in monitoring for the recurrence of bladder cancer.

B. Investigators

Sensitivity Monitoring Trial 9201 was conducted at 14 different geographical sites. (See Table 5.) Investigators were chosen based on their credentials, experience, facilities, personnel support and anticipated number of eligible patients. All geographic regions are well-represented and institutions ranged in size from large academic centers to small private group practices.

TABLE 5 **Sensitivity Monitoring Trial 9201**

| | NAME AND ADDRESS OF INVESTIGATOR | SUBJECTS ENROLLED | SAMPLES ENROLLED |
|---|---|---|---|
| 1. | Gerald Chodak, M.D.<br>University of Chicago<br>Medical Center<br>Chicago, IL | 13 | 18 |
| 2. | Paul F. Schellhammer, M.D.<br>Sentara Cancer Institute<br>Norfolk, VA | 49 | 95 |
| 3. | Mark Jarowenko, M.D.<br>Hershey Medical Center<br>Hershey, PA | 41 | 90 |

| | | | |
|---|---|---|---|
| 4. | Bob Johnson, M.D.<br>Urology Surgical Associates<br>Springfield, MO | 23 | 40 |
| 5. | Mark Soloway, M.D.<br>University of Miami<br>Miami, FL | 55 | 100 |
| 6. | Joel Sheinfeld, M.D.<br>Memorial Sloan Kettering<br>Cancer Center<br>New York, NY | 53 | 117 |
| 7. | Jay Patel, M.D.<br>St. Louis, MO | 17 | 47 |
| 8. | William J. Ellis, M.D.<br>Seattle VA Medical Center<br>Seattle, WA | 32 | 52 |
| 9. | Edward Schervish, M.D.<br>St. John's Professional<br>Detroit, MI | 15 | 32 |
| 10. | Ralph de Vere-White, M.D.<br>University of California-Davis<br>Sacramento, CA | 55 | 135 |
| 11. | Michael F. Sarosdy, M.D.<br>University of Texas Health<br>Science Center<br>San Antonio, TX | 71 | 138 |
| 12. | Edward Messing, M.D.<br>University Hospital & Clinics,<br>Madison, WI | 29 | 65 |

| | | | |
|---|---|---|---|
| 13. | M'Liss Hudson, M.D.<br>Washington University<br>St. Louis, MO | 50 | 115 |
| 14. | Donald L. Lamm, M.D.<br>West Virginia University<br>Morgantown, WV | 11 | 25 |
| | TOTALS | 514 | 1069 |

C. **Population Studied**

The study population consisted of patients with a confirmed diagnosis and history of bladder cancer, of various stages and grades. All patients had been diagnosed to have bladder cancer for varying lengths of time prior to the study. Multiple urological examinations were conducted over the course of the study. Since standard medical practice is to include a cystoscopy and VUC, urine samples were split for analysis of VUC and for the BTA® test. The BTA® test was performed blind at each routine visit, before cystoscopy was routinely performed, to determine correlation with the cystoscopy results. No clinical decisions were made based on the BTA® test result.

Inclusion criteria for Sensitivity Monitoring Trial 9201 included patients of any race, gender, and age with histologically proven TCC of the bladder or upper urinary tract being monitored for bladder cancer recurrence by cystoscopy who gave informed consent. Patients were excluded from Sensitivity Monitoring Trial 9201 if they had:

- received systemic chemotherapy or intravesical therapy within one month,
- received radiation therapy for bladder cancer within 3 months,
- received any investigational drug within 7 days,
- undergone urinary tract surgery (e.g., cystoscopy, biopsy, resection), in-dwelling urinary catheterization or lithotripsy within 14 days,
- any genitourinary malignancy currently under treatment,
- failed to provide at least 25 ml of voided urine for testing,
- not voided since any ejaculation (males) or sexual intercourse, and prior to voided urinary sample for BTA testing, or
- failed to give informed consent.

Urine samples from 514 patients with bladder cancer representing 1069 visits were evaluated at 14 centers located throughout the United States. Fifteen subjects comprising fifty-five visits were eliminated from the database due to the following protocol violations: 1) Three subjects had no cytology performed. 2) Six subjects had treatment or bladder trauma less than 14 days before sampling; and 3) One patient had two visits, both of which were excluded for one or the other above reasons. In addition five subjects had other reasons for exclusion: 1) BTA and cytology were not performed on the same sample (2), 2) no confirmed history or diagnosis of bladder cancer (2) [suspicious upper tract diagnosis not confirmed (1)], and 3) BTA results indeterminate because of severe gross hematuria.

There remained 1014 evaluable visits and 499 evaluable patients. The 499 evaluable patients had an age range of 31

to 95 years with a mean age of 69.1 years. The population was 90.8 percent Caucasian, 7.0 percent Other (Asian, Hispanic, Other), and 2.2 percent black. There were 398 men and 101 women in the study. None of the fourteen (14) institution contributed more than 14 percent of the subjects.

## D. Results of Sensitivity Monitoring Trial 9201

### 1. BTA® Diagnostic Performance Characteristics Per Visit

Disease positive, suspicious, and negative diagnoses were recorded at 205 (20 percent), 28 (3 percent), and 781 (77 percent) of 1014 patient visits. (See Table 6.) Disease-suspicious results were counted as disease negative results since only five of the 28 suspicious cases were biopsy confirmed; all five of these were biopsy confirmed to be disease negative. The diagnostic performance characteristics of the BTA® test on a per visit basis can be seen in Table 6.

**Table 6.**

Sensitivity/Specificity Calculation Per Visit
TCC Suspicious with Negative Disease

| | | DISEASE Pos | DISEASE Neg | |
|---|---|---|---|---|
| BTA | Pos | 89 | 156 | 245 |
| | Neg | 116 | 653 | 769 |
| | | 205 | 809 | 1014 |

Sensitivity= 89/205 = 43%
Specificity= 653/809 = 80.7%
Predictive Value Positive = 89/245 = 36.3%
Predictive Value Negative = 653/769= 85%
Prevalence of Recurrence = 205/1014 = 20.2%

Many patients had more than one visit during the monitoring trial. Subject visits ranged from 1 to 5 (mean 2.0). Some patients had one, two, and three negative visits, one, two, or three positive visits, or a mixture of positive, negative, and suspicious visits. To avoid bias caused by counting each patient a variable number of times, the test performance characteristics were calculated also on a per patient basis.

2. **BTA® Diagnostic Performance Characteristics Per Patient**

Of the 499 evaluable patients monitored prospectively for bladder cancer, 151 had at least one monitoring visit where TCC was diagnosed by cystoscopy with or without biopsy. To calculate test sensitivity, the sponsor counted the first disease positive visit for each patient. (see Table 7.)

**Table 7**

Sensitivity/Specificity Calculation Per Patient
First Positive/First Negative Visit
TCC Suspicious with Negative Disease

| | | DISEASE Pos | DISEASE Neg | |
|---|---|---|---|---|
| BTA | Pos | 61 | 69 | 130 |
| | Neg | 90 | 279 | 369 |
| | | 151 | 348 | 499 |

Sensitivity= 61/151 = 40%
Specificity= 279/348 = 80.2%
Predictive Value Positive = 61/130 =47%
Predictive Value Negative = 279/369= 75.6%
Prevalence of Recurrence = 151/499 = 30.8%

The diagnostic specificity of the BTA® test relative to cystoscopy was 80 percent calculated on a per visit or per patient basis.

3. **Predictive Values**

Positive and negative predictive values and monitoring specificity were calculated using Bayes formula. Table 8 lists the sensitivity and specificity in a monitoring population as well as positive and negative predictive values at a 10 percent prevalence rate.

**TABLE 8 Sensitivity/Specificity Monitoring**

| Test | Monitoring Sensitivity | Monitoring Specificity | Positive Predictive Value | Negative Predictive Value |
|---|---|---|---|---|
| BTA® | 40.3 | 80.3 | 18.4 | 92.3 |
| VUC | 19.4 | 95.9 | 34.5 | 91.5 |

4. **Comparison of BTA® Performance Characteristics To VUC**

Method comparison between the BTA test and voided urine cytology (VUC) was examined also in the Sensitivity Monitoring Trial 9201. Results of 151 evaluable visits included 37 VUC suspicious (VUCsus) results. During review of the PMA, the question arose regarding whether VUC suspicious results should be considered as positive or negative VUC results. It was decided that the method comparison between the BTA test and VUC results should be considered both ways.

When VUCsus results were counted as negative for VUC, the BTA® test had a sensitivity per patient of 40

percent (95 percent CI, 32.5 - 48.7) compared to 17 percent for VUC (95 percent CI, 11.0 - 23.5) ($p < 0.001$)(See Table 9.) On the other hand, if VUCsus results were grouped with the positive VUC results, the two tests had equal sensitivities (40 percent versus 41 percent, p= 1.000) (See Table 10). (However, the concomitant specificity of VUC did decrease from 96.8 percent to 91.5 percent when VUCsus results were counted as positive VUC results).

**Table 9: Trial 9201, Sensitivity by Combined Stage and Grade Groupings (First Positive Visit), VUC Suspicious Results = Negative VUC Results**

| Stage and Grade | Visit Count | Test Name | Sensitivity (%) | 95% Confidence Interval | p Value |
|---|---|---|---|---|---|
| ALL | 151* | BTA<br>VUC<br>BTA + VUC | 40<br>17<br>48 | 32.5 - 48.7<br>11.0 - 23.5<br>39.5 - 56.0 | < 0.001 |
| Ta I/II<br>(low risk of progression) | 61 | BTA<br>VUC<br>BTA + VUC | 30<br>3<br>31 | 18.5 - 42.6<br>0.4 - 11.4<br>19.9 - 44.3 | < 0.001 |
| Ta III, T1 II/III<br>(high risk of progression) | 32 | BTA<br>VUC<br>BTA + VUC | 47<br>19<br>56 | 29.1 - 65.3<br>7.2 - 36.4<br>37.7 - 73.6 | 0.035 |
| ≥ T2 | 14 | BTA<br>VUC<br>BTA + VUC | 64<br>43<br>71 | 35.2 - 87.2<br>17.7 - 71.1<br>41.9 - 91.6 | 0.375 |
| Tis III | 9 | BTA<br>VUC<br>BTA + VUC | 56<br>56<br>78 | 21.2 - 86.3<br>21.2 - 86.3<br>40.0 - 97.2 | 1.00 |

* Positive cystoscopy results without stage and/or grade information are included in this total.

Conclusion: In patients with Ta I/II, Ta III, T1 II/III tumors, BTA is significantly more sensitive than VUC ($p < 0.001$ and 0.035, respectively). In patients with tumors Tis III and ≥ T2 tumors, BTA sensitivity is greater or equal to VUC, but the difference is not statistically significant. If either BTA or VUC is positive (combined use), the sensitivity improved in Tis III and ≥ T2 tumors, although the sample size is too small for statistical significance to be achieved.

**Table 10: Trial 9201, Sensitivity by Combined Stage and Grade Groupings (First Positive Visit), VUC Suspicious Results = Positive VUC Results**

| Stage and Grade | Visit Count | Test Name | Sensitivity (%) | 95% Confidence Interval |
|---|---|---|---|---|
| ALL | 116 | BTA<br>VUCsus<br>BTA + VUCsus | 41<br>44<br>61 | 31.5 - 50.0<br>34.8 - 53.5<br>51.7 - 70.1 |
| Ta I<br>(low risk) | 29 | BTA<br>VUCsus<br>BTA + VUCsus | 17<br>17<br>31 | 5.8 - 35.8<br>5.8 - 35.8<br>15.3 - 50.8 |
| Ta II<br>(low risk) | 32 | BTA<br>VUCsus<br>BTA + VUCsus | 41<br>25<br>53 | 23.7 - 59.4<br>11.5 - 43.4<br>34.7 - 70.9 |
| Ta III, T1 II/III<br>(high risk) | 32 | BTA<br>VUCsus<br>BTA + VUCsus | 47<br>63<br>78 | 29.1 - 65.3<br>43.7 - 78.9<br>60.0 - 90.7 |
| ≥ T2 | 14 | BTA<br>VUCsus<br>BTA + VUCsus | 64<br>71<br>86 | 35.2 - 87.2<br>41.9 - 91.6<br>57.2 - 98.2 |
| Tis III | 9 | BTA<br>VUCsus<br>BTA + VUCsus | 56<br>89<br>89 | 21.2 - 86.3<br>51.7 - 99.7<br>51.7 - 99.7 |

Conclusion: There is no significant difference between BTA and VUCsus sensitivity when broken out by tumor stage and grade groups. If either BTA test or VUCsus is positive (combined use), the sensitivity significantly improves ($p<0.001$). Cases (35) not falling into the above stage and grade groupings were excluded either because biopsy was not done or because biopsy information was incomplete.)

As can be seen in Tables 9 and 10, both the BTA test and VUC increase in sensitivity as stage and grade of the recurrent cancer increase.

When VUCsus were counted as negative results (See Table 9), the BTA® test was more sensitive than VUC in bladder cancers with low and high risk of progression. In 14 patients with invasive cancer greater than or equal to transitional cell carcinoma Stage 2 (T2), and 9 patients with Tis Grade III, the sensitivities were not significantly different.

On the other hand, if VUCsus are counted as positive (See Table 10), there were no significant differences between the BTA® results and the VUC results, with the exception of TIS Grade III. In patients with TIS Grade III if VUCsus are counted as positive, VUC had better sensitivity than the BTA® test (89 percent vs. 56 percent in 9 cases).

In all cases, the two tests show independence. Sensitivities correlating with bladder cancer were better when the two tests were used in combination than when either test was used alone.

## VIII. Conclusions Drawn from the Studies

The BTA® test demonstrated the ability to detect the presence of Bladder Tumor Associated Analytes in human urine to aid in the management of bladder cancer patients.

Preclinical studies demonstrated reactivity of the BTA® test with BTA. Performance evaluation of the test met all design specifications, including limits of detection, interfering

substances, high dose hook effect, sample dilution, sample centrifugation, sample stability studies, reproducibility, kit stability, and kit shipping conditions.

Acceptable specificity of the test in populations of persons with no history of bladder cancer was demonstrated in Specificity Trial 9301. Test specificity in apparently healthy individuals and subjects without genitourinary disease or cancer was 96 percent. It was 93 percent in subjects with genitourinary disease or cancer. (See Table 4.) Any persons that had trauma to the bladder or leukocytes in the urine by dipstick were removed from these groups.

Excessive false positive results were seen in the group of persons with leukocytes in the urine (positive on urinalysis dipstick) (48 percent false positive results) and in the group of persons with trauma to the urinary tract within 14 days prior to having the BTA® test performed (41 percent false positive results) (See Table 4).

The results of the Sensitivity Monitoring Trial 9201 evaluated the qualitative clinical performance characteristics of the BTA® test compared to cystoscopy. The diagnostic specificity in the intended use population of persons monitored for bladder cancer recurrence was 80 percent calculated either per patient or per visit. The BTA® test had a 40 or 43 percent diagnostic sensitivity per patient or per visit, respectively.

In the same trial, method comparison of VUC versus the BTA® test showed that VUC diagnostic specificity compared to the reference method of cystoscopy is better than that of the BTA® test (>91.5 percent versus 80 percent).

On the other hand, the BTA® test had greater diagnostic sensitivity compared to the cystoscopy reference method than VUC (40 percent versus 17 percent) when VUCsus results were considered to be negative. When VUCsus results were considered to be positive, the sensitivity of the two tests was equivalent at 40 percent.

The Sensitivity Monitoring Trial 9201 also showed that the two tests were independent indicators of bladder cancer recurrence. Diagnostic sensitivity for bladder cancer recurrence increased when both tests were used in combination (see Tables 9 and 10).

CDRH has concluded that the device is reasonably safe and effective when used as intended for the qualitative measurement of BTA in human urine to aid in the management of bladder cancer patients.

## IX. Panel Recommendation

The Immunology Devices Panel recommended at the panel meeting September 21, 1995 that the PMA for the BTA® test be approved with no conditions.

## X. CDRH Action on the Application

CDRH concurred with the recommendation of the Panel. CDRH issued an approvable letter to the applicant on November 7, 1995 requesting submission of yearly follow-up in the annual reports. An approval letter was issued to Bard® Diagnostic Sciences, Inc. on November 29, 1995.

The applicant's manufacturing and control facilities were inspected on September 7, 1995, and the facilities were

found to be in compliance with the Good Manufacturing Practice (GMP) Regulations.

## XI. Approval Specifications

Directions for Use: See attached labeling.

Conditions of Approval: None

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# Bard® BTA®

**Bard**
**Diagnostic Sciences**

## A Rapid Urine Test
for the Detection of Bladder Tumor Associated Analytes

**Test kit No: 662115 (15 Patient Determinations)**
**Test kit No: 662130 (30 Patient Determinations)**

*Caution: U.S.A. Federal law restricts this device to sale and distribution by or on the order of a physician, or to a clinical laboratory and use is restricted to by or on the order of a physician.*

## INTENDED USE

The Bard BTA rapid latex agglutination test is an *in vitro* device intended for the qualitative measurement of Bladder Tumor Associated Analytes in human urine to aid in the management of bladder cancer patients.

## SUMMARY AND EXPLANATION OF THE TEST

**Bladder Cancer**

Bladder cancer is the fourth most common form of cancer in men and the eighth most common form in women in the United States.[1] Patients with previous diagnosis of bladder cancer are routinely followed for recurrence by urine cytology and routine cystoscopy. Both cystoscopy and cytology have their limitations.

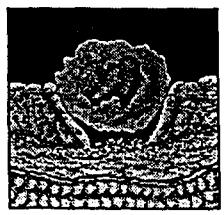
Recent research[2] indicates that the BTA test, which detects Bladder Tumor Associated Analytes in urine of some bladder cancer patients may be a useful adjunct in the management of bladder cancer patients. Positive BTA test results were independent of cytology results. Optimum sensitivity for the detection of recurrence of bladder cancer was seen when results of both tests were considered.

**Bladder Tumor Associated Analytes**

Bladder Tumor Associated Analytes were isolated from the urine of some patients with bladder cancer. They are not detected in urine of most normal persons and persons with other diseases. They have been shown to contain high molecular weight polypeptides of molecular weight 16 to 165 kD. Immunologically they appear to consist of complexes of basement membrane proteins and in some cases may also contain Immunoglobulin G (IgG).

Three causes for the appearance of Bladder Tumor Associated Analytes in urine of some bladder cancer patients have been postulated: (1) invasion of the basement membrane; (2) production by the tumor itself; and (3) a combination of these which may be linked with the body's immune response.

**(1) INVASION OF BASEMENT MEMBRANE**



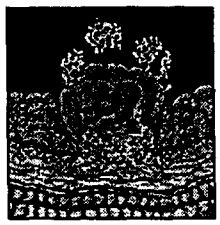
Three stages have been seen in tumor invasion.

**a) Inducement**

Tumor cells induce the epithelial cells lining the bladder to retract, exposing an extracellular matrix known as the basement membrane. Composed of Type IV collagen, fibronectin, laminin and proteoglycans, this extracellular matrix surrounds the mucosal lining, separating it from the submucosal tissues.[3,4,5]

**b) Attachment**

Tumor cells attach to the basement membrane through a biochemical process involving the production and secretion of endogenous basement membrane proteins. These proteins bind to surface receptors on the membrane and anchor the tumor.



**c) Release of Basement Membrane Complexes**

Bladder tumors have been shown to secrete proteolytic enzymes that degrade the basement membrane into fragments of its basic components, e.g., Type IV collagen, fibronectin, laminin and proteoglycans.[6,7,8,9] These components were discharged into the urine where they combine to form basement membrane complexes.

Basement membrane complexes have been detected and characterized in urine as a means to detect tumors in the bladder. The loss of basal lamina proteins in the case of bladder cancer leads to the formation of detectable protein complexes in urine, which reflect the tumor's invasive process.[10]

**(2) PRODUCTION BY THE TUMOR ITSELF**

Some bladder tumors which do not appear to be invasive were positive with the BTA test. This supports the observation that bladder tumor cells produce and secrete basement membrane proteins during the attachment phase prior to actual tumor invasion.

**(3) IMMUNE RESPONSE**

Cross-reactivity studies revealed that the BTA test was positive at elevated levels of IgG. Thus, the BTA test may have a cross-reactivity with IgG that could aid in the detection of Bladder Tumor Associated Analytes in some patients experiencing concomitant antibody response.

Bladder Tumor Associated Analytes have been detected in the urine of some patients with bladder cancer. Recent research indicates that the BTA test, which detects Bladder Tumor Associated Analytes can be a useful tool in the management of recurrent bladder cancer.

## PRINCIPLE OF THE PROCEDURE

The Bard BTA test is a latex agglutination assay for the qualitative detection of Bladder Tumor Associated Analytes in urine. Samples of urine from patients with a history of bladder cancer are mixed with latex particles coated with non-specific human IgG and blocking agents. If the Bladder Tumor Associated Analytes are present in urine at a significant level, they will combine with the latex particles to produce an agglutination reaction. Following the formation of the agglutinates, a visual color change differentiates positives from negatives by use of a specially prepared Test Strip. Urine samples are considered positive when a clear yellow

color is observed above a blue band of agglutinate on the Test Strip and negative when the Test Strip is green or green above blue without a yellow color. Positive and Negative Controls are supplied along with a visual guide for Test Strip interpretation.

## CONTRAINDICATIONS

- Do not use kit components after expiration date.
- Do not use kit components from other lots of this test.
- Do not reuse disposable Test Stations, Test Strips, droppers, microtubes, or pipette tips. Discard after single use.
- Do not touch the pad portion of the Test Strip or use Test Strips that are damaged.
- Do not use test kits that are delivered damaged or that show leakage from reagent vials.

## WARNINGS

- **For *in vitro* diagnostic use.**
- Buffer, Controls and Reagent each contain 0.1% sodium azide which, if allowed to accumulate, can form explosive compounds in lead and/or copper plumbing. When disposing of Buffer, Controls, or Reagent, flush disposal area with large volumes of water to prevent possible formation of such explosive compounds.
- Human source material used for the preparation of the BTA Reagent was tested and found to be negative for antibodies to Human Immunodeficiency Virus Type 1 (HIV-1), for Hepatitis B Surface Antigen (HBsAg) and for nucleic acid sequences characteristic of Human Immunodeficiency Virus Type 2 (HIV-2) and Hepatitis C Virus (HCV). Human source material used for the preparation of the Positive Control was heat/low pH inactivated. No available test can offer absolute assurance of the absence of infectious agents. Handle these reagents and all materials coming into contact with them as potentially infectious.

## PRECAUTIONS

- To avoid cross-contamination of samples, use a new dropper, tube, and pipette tip for each urine sample.
- Wear disposable gloves. Wash hands thoroughly after handling specimens and kit contents.

## SOLUTIONS AND REAGENTS

- **Buffer (B)**
  2.7 ml in a Dropper Bottle. HEPES buffer with 0.1% sodium azide as a preservative.
- **Negative Control (-)**
  3.0 ml in a Dropper Bottle. Saline and glycine buffer with 0.1% sodium azide as a preservative.
- **Positive Control (+)**
  3.0 ml in a Dropper Bottle. Human collagen IV (approximately 20µg /ml) in saline and glycine buffer with 0.1% sodium azide as a preservative.
- **Bard BTA Reagent (R)**
  3.3 ml in a Screw Cap Bottle. Polystyrene latex particles (human IgG coated), with blocking agent and 0.1% sodium azide as a preservative.

## STORAGE AND STABILITY

- The BTA test kit (opened or unopened) is stable until the expiration date indicated in labeling when the BTA Reagent and Solutions are stored refrigerated at 2-8°C. All remaining materials (disposables and accessories) can be stored at room temperature (15-30°C). Keep all materials dry.
- Reagents can be used immediately after removal from refrigerated storage. Return reagents to refrigeration after use.
- Do not freeze the BTA Reagents or other solutions.

## INDICATIONS OF REAGENT AND CONTROL DETERIORATION

When mixed, the Reagent (R) should be a milky green suspension. The Positive (+) and Negative (-) Controls should be clear, pale yellow solutions and the Buffer (B) Solution should be clear. They should be free of gross particulate matter. If turbidity is evident, the components should not be used.

## SPECIMEN COLLECTION, STORAGE, PREPARATION

Voided urine or urine from a catheterized patient is required for the BTA test. Bladder barbotage specimens, serum, plasma or whole blood should not be used. Urine should be collected without preservatives in a clean, dry urine cup and must be tested within 48 hours of collection. If urine is to be used for other tests, separate out a portion of specimen (a minimum of 2 ml) for this test to avoid contamination. Mix urine before testing. Label the urine sample appropriately. If the urine sample is not to be tested at the time of collection, it should be refrigerated (2-8°C) until tested. Test refrigerated urine within 48 hours after collection. If the urine sample is more than 48 hours old, discard and obtain a fresh sample.

- Do not use paper or foam cups for urine specimen collection or storage.
- Do not test urine specimens that have been heated or frozen.
- Do not use samples from timed urine collections (24 hour urines).
- Avoid testing urine with elevated levels of leukocytes in the urine (positive urinalysis test strip reading).
- The effects of radiation therapy within three months or systemic chemotherapy within 30 days on the BTA test is unknown. Testing prior to these time frames is not recommended.
- BTA testing should not be performed in patients until at least 30 days after intravesical therapy. The following intravesical therapies were evaluated: BCG, mitomycin C, Thiotepa, bropiramine (investigational) and interferon (investigational). Patients receiving intravesical agents other than those evaluated should be tested (after 30 days) at the discretion of the physician.
- The effects of experimental drugs on the BTA test are unknown. Patients taking an experimental or investigational drug should not be tested until the drug has been fully excreted.
- Semen is known to cause false positive BTA test results. First voided urine following ejaculation should not be tested. This is a particular problem after some cases of transurethral resection of the prostate where retrograde ejaculation may occur.
- For trauma to the bladder or urinary tract due to surgery, biopsy, etc., the physician should allow ample time for trauma recovery before using the test.

## CONTENTS OF THE KIT

- Buffer (B) in a Dropper Bottle
- Negative Control (-) in a Dropper Bottle
- Positive Control (+) in a Dropper Bottle
- Bard BTA Reagent (R) in a Screw Cap Bottle
- 15 Disposable Test Stations (Test Kit No. 662115)
  30 Disposable Test Stations (Test Kit No. 662130)
- 15 Disposable Test Strips (Test Kit No. 662115)
  30 Disposable Test Strips (Test Kit No. 662130)
- 1 Product Insert with Directions for Use
- 1 Quick Reference Card with Illustrated Test Directions and Interpretation Guide

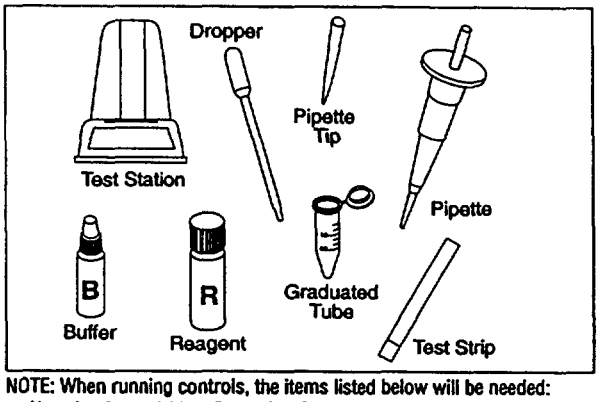
## MATERIALS REQUIRED BUT NOT PROVIDED

- Timer
- Disposable Gloves
- Disposable Droppers
- Graduated Microtubes with Caps (graduated to at least 0.5 ml)
- Pipette capable of delivering 35 µl
- Pipette Tips

NOTE: An Accessory Kit (Catalog No. 662110) which includes droppers, graduated microtubes, pipette, and pipette tips is available from Bard.

## MATERIALS FOR PATIENT SAMPLE TEST PROCEDURE

- 1 Disposable Test Station
- 1 Disposable Dropper
- 1 Graduated Tube with Cap
- 35 µl Pipette and Tips
- 1 Test Strip
- Buffer (B) in Dropper Bottle
- Bard BTA Reagent (R)
- Quick Reference Card with Interpretation Guide




NOTE: When running controls, the items listed below will be needed:

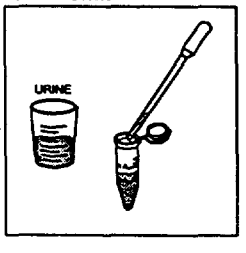
- Negative Control (-) in Green Cap Dropper Bottle
- Positive Control (+) in Red Cap Dropper Bottle
- 1 Disposable Test Station for each control
- 35 µl Pipette and Tips
- 1 Test Strip for each control
- Bard BTA Reagent (R)
- Quick Reference Card with Interpretation Guide

## REAGENT PREPARATION

Buffer, Controls, urine sample and Bard BTA Reagent can be used immediately upon removal from refrigerated storage or after reaching room temperature. Shake/mix Bard BTA Reagent well before use.

## PATIENT SAMPLE TEST PROCEDURE

### 1. ADD URINE

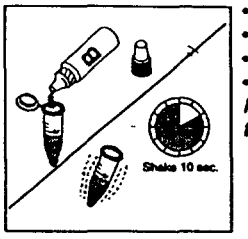



*Use new dropper (not pipette) and graduated tube labeled with patient identifier.*

- Squeeze dropper bulb. Lower end into urine. Let go of bulb to fill dropper.
- Hold tip just above graduated tube. Carefully squeeze bulb and add urine up to the 0.5 ml line on tube.
- Throw dropper away. Use new dropper and tube for each sample.
- The dropper is used only for this step.

NOTE: Urine should be less than 48 hours old.

### 2. ADD BUFFER/SHAKE

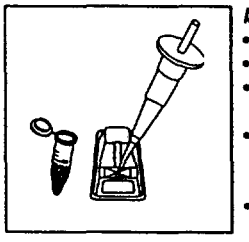



*Hold bottle upside down while adding buffer.*

- Add ONE drop of Buffer (B) to tube.
- Put cap on tube.
- Shake tube for at least 10 seconds.
- Open tube.

*NOTE: Urine must be tested within 30 minutes after adding buffer.*

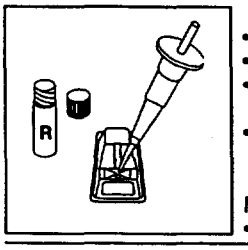
### 3. ADD URINE TO TEST WELL



*Put new Test Station upright on a flat surface and label with patient identifier.*

- Push new tip onto 35 µl pipette until tight.
- Push plunger all the way down on pipette.
- Lower tip into urine. Let plunger slowly rise to draw urine into the tip.
- Hold tip above test well. Push plunger down to add all the urine in the tip. Do not add any more urine to well.
- Remove and discard pipette tip.

### 4. ADD REAGENT TO WELL

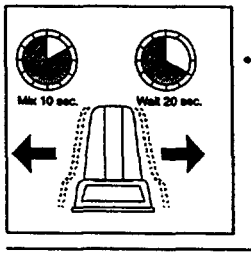



- **Gently shake Reagent (R) for 10 seconds before use.**
- Push new tip onto 35 µl pipette until tight.
- Push plunger all the way down.
- Lower tip into well mixed Reagent (R). Let plunger slowly rise to draw reagent into tip.
- Hold tip above test well. Tip should not touch anything. Push plunger down to add all of reagent from tip into test well.

NOTE: Add Reagent to test well only one time.

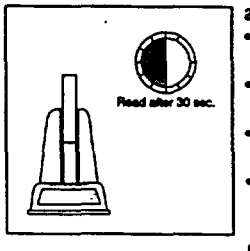
- Remove and discard pipette tip.

### 5. MIX




- Without splashing, mix vigorously for 10 seconds by sliding station side-to-side across a flat surface.
- Wait at least 20 seconds, but no longer than 5 minutes before adding Test Strip.

### 6. ADD TEST STRIP




Make sure paper pads on Test Strips are smooth and still attached to the plastic backing.

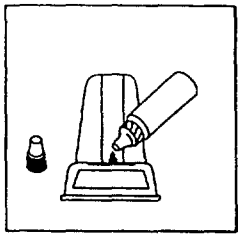
- Hold Test Strip with paper pad down and facing towards you.
- Slowly lower into test well. Rest Test Strip in groove of Test Station and leave in well.
- Wait at least 30 seconds but no longer than 5 minutes before reading.
- Remove Test Strip from test well and compare to the Interpretation Guide found on the Quick Reference Card.

NOTE: See "Interpretation of Results" section in this insert for explanation of test and control results.

- Discard graduated tube of buffered urine after 30 minutes.

## CONTROL TEST PROCEDURE

**1. ADD CONTROL TO TEST WELL**



Use a new Test Station for each control. When Controls are run, always run both a Negative and a Positive Control.

*Hold dropper upside down while adding control.*

- Place 2 NEW Test Stations upright on flat surface. Label + or -.
- Carefully squeeze ONE drop of Negative Control (green cap) into the Test Station well labeled "-" and ONE drop of Positive Control (red cap) into the other Test Station well labeled "+". NOTE: Do not splash.
- Go to step 4 of PATIENT SAMPLE TEST PROCEDURE (ADD REAGENT TO WELL)
- Perform steps 4, 5 and 6 as in Patient Sample Test Procedure.

## INTERPRETATION OF RESULTS

The BTA test reaction uses a combination of dyes to produce a unique color development for positive and negative test results. Please refer to the Interpretation Guide on the Quick Reference Card for visual examples and an explanation of positive and negative Test Strip pad colors.

A positive test MUST HAVE a YELLOW color at the TOP of the test pad. A negative test will have a GREEN or LIGHT GREEN color at the TOP of the test pad. Ignore any blue color at the bottom of the test pad.

## TROUBLESHOOTING

***STREAKS***

Streaks can be caused by insufficient mixing of Reagent with buffered urine or Control. If streaks of color appear on the Test Strip, repeat the entire test for specimen, Negative Control and Positive Control to ensure accuracy.

***EXCESS FLUID IN WELL AT END OF TEST***

If the test well appears more than one third full and the Test Strip from that well is saturated as compared to other Test Strips, excess fluid should be suspected. Repeat the entire test for specimen, Negative Control and Positive Control to ensure accuracy. It is normal for a small amount of fluid to remain in the well when the test is completed.

***INSUFFICIENT FLUID IN TEST WELL***

If the top of the test pad remains white at the end of the test procedure, insufficient fluids in the test well should be suspected. Repeat the entire test as described above.

***QUALITY CONTROL GIVES INCORRECT RESULTS***

The BTA Test Strip pad using the Negative (–) or Positive (+) Control should look like the corresponding Test Strip pads in the Interpretation Guide. If either the Positive or Negative Control tests do not give the correct result, carefully read the instructions explained in the Control Test Procedure section of the insert and repeat the test with both Controls. After repeating the tests, if results are still not correct for either or both the Positive and Negative Controls, then the Bard BTA Test kit should not be used for reporting patient results. Patient results should only be reported if both the Positive and Negative Controls provide the correct results. If problems continue, contact Bard Urological at 1-800-526-4455 for assistance.

## LIMITATIONS

Results of the BTA test should not be interpreted as absolute evidence for the presence or absence of TCC of the bladder. Elevated levels may be observed in urine from patients with recent surgery, biopsy or other invasive trauma to the bladder or urinary tract. Active infections of the genitourinary tract, renal or bladder calculi, and positive leukocyte reading on urinalysis test strip may cause false positive test results. TCC of the kidney or ureters may give a positive BTA test result. The result from the BTA test should be used only in conjunction with information available from the clinical evaluation of the patient and other diagnostic procedures. The BTA test should not be used as a screening test.

## QUALITY CONTROL

Good laboratory practices recommend the use of control specimens with each assay run to ensure that all reagents and protocols are performing properly. Consult your state and federal regulations and other appropriate accrediting organizations for quality assurance protocols if you do not have an internal quality assurance program for your facility. The BTA test kit contains Controls that can be used to verify that all the BTA test kit reagents, with the exception of Buffer (B), are functioning correctly. The control tests should produce the appropriate result when compared to the Quick Reference Card. If either Control does not give the correct result, patient results should not be reported. See Troubleshooting section. One Negative and one Positive Control test should also be run with each new lot of BTA test kits you receive.

Note: the Controls included in the kit will not detect an error in performing the patient sample dilution and testing procedure.

## EXPECTED RESULTS

***SPECIFICITY***

BTA test specificity was determined in multicenter clinical studies. BTA test specificity in normal healthy persons and persons with non-genitourinary diseases and malignancies was studied in 646 non-bladder cancer patients in Washington and Alabama. The average age was 53 years and 52% were males. The normal healthy population consisted of smokers and non-smokers.

The non-genitourinary diseases included diabetes, arthritis, lupus and other collagen degenerative diseases, and malignancies included mammary, pulmonary, gastrointestinal carcinomas and leukemia and lymphomas. The GU cancers (non-bladder GU cancer) category consisted of prostate, renal cell, endometrial, ovarian and other genitourinary carcinomas. The GU disease category consisted of patients with prostatitis, urethritis, stones, urinary tract infections, incontinence, sexually transmitted diseases and other disorders.

The results indicated that in healthy individuals (smokers and non-smokers) and individuals without GU diseases and malignancies, the BTA test negative rate was 96%. False positive BTA test results may occur in as many as 48% of individuals with a positive leukocyte reading by urinalysis test strip.

In a multicenter study conducted in 14 different geographic locations throughout the United States, the BTA test specificity was 80% in 348 patients with a history of bladder cancer being monitored for recurrence with no evidence of disease.

Expected results may vary depending on the patient population tested.

## BTA TEST SPECIFICITY RESULTS

| Patient Type | Number of Subjects | Test Negative(%) |
|---|---|---|
| **History of Transitional Cell Carcinoma (No Evidence of Disease)** | 348 | 80% |
| **Healthy Subjects, Non-Genitourinary (GU) Diseases and Cancers** (Urine negative for leukocytes) | 303 | 96% |
| Healthy Non-Smokers | 123 | 96 |
| Healthy Smokers | 82 | 95 |
| Non-GU Diseases | 49 | 94 |
| Non-GU Cancers | 49 | 100 |
| **GU Diseases and Cancers (Non-Bladder)** (Urine negative for leukocytes) | 232 | 93% Total |
| Benign Prostatic Hyperplasia | 35 | 94 |
| Benign Renal Disease | 28 | 89 |
| Misc. GU Disease | 47 | 94 |
| Sexually Transmitted Diseases | 50 | 90 |
| Prostate/Renal Cancers | 40 | 95 |
| Other GU Cancers (Non-bladder) | 32 | 94 |
| **GU Trauma ≤ 14 Days** | 59 | 59% |
| Leukocyte Negative‡ | 40 | 75 |
| Leukocyte Positive‡ | 18 | 22 |
| Leukocyte Unknown‡ | 1 | 100 |
| **Urine Positive for Leukocytes** (All patient types except Trauma category) | 46 | 52% |
| Urine Leukocyte Status Unknown (All categories except Trauma) | 6 | 83% |
| TOTAL | 994 | |

‡ Leukocyte positive by urinalysis test strip

## SENSITIVITY

Bard BTA test sensitivity results were determined in a multicenter study, where 499 patients were monitored prospectively for bladder cancer with the BTA test and voided urine cytology (VUC) at the same time as cystoscopy. Results of both test methods are presented below for the 151 patients who had a recurrence (suspicious VUC results were counted as negative VUC results):

| Stage & Grade at Diagnosis | Number of Subjects | Positive BTA (%) | Positive VUC (%) | Positive BTA + VUC (%) |
|---|---|---|---|---|
| Ta G I/II | 61 | 30 | 3 | 31 |
| Ta G III, T1 G II/III | 32 | 47 | 19 | 56 |
| T2, T3, T4 | 14 | 64 | 43 | 71 |
| TIS G III | 9 | 56 | 56 | 78 |
| Positive cystoscopy only | 35 | 40 | 17 | 51 |

The BTA test was shown to be more sensitive than VUC in all categories except for TIS G III where it was identical in sensitivity when using VUC results reported as positive for malignancy. Optimum sensitivity is achieved in high stage and grade disease when both VUC and the BTA test are performed in combination. If a suspicious VUC is considered positive, the results are comparable for all stages and grades.

## PERFORMANCE CHARACTERISTICS

### *LIMITS OF DETECTION*

The sensitivity of the BTA test was determined to be 9.8 µg/ml of Bladder Tumor Associated Analytes by dilution of a patient sample containing 2500 µg/ml Bladder Tumor Associated Analytes and approximately 9 µg collagen IV/ml calculated by standard curve analysis. (Human placental collagen IV is used as the standard and control material in the test, and was used in the optimization of the assay).

### *HIGH DOSE HOOK EFFECT*

High dose hook (prozone) effect tests were conducted to determine if the BTA test is free from interference from high concentration positive patient samples. Results showed that there was no prozone effect up to 4000 µg/ml type IV collagen and 2500 µg/ml Bladder Tumor Associated Analytes in a patient's urine sample, which was the highest concentration available for testing.

### *REPRODUCIBILITY*

Three lots of Bard BTA Reagent were used for the reproducibility studies to determine between-day, within-day and lot-to-lot variability. These studies were conducted by testing 14 different samples (11 samples were run with 10 replicates, 3 samples were run with 5 replicates) per day for five days. Between laboratory qualitative reproducibility studies were conducted at six laboratories by testing one lot of Bard BTA Reagent using 14 different samples run 10 times on one day. All reproducibility studies showed near total qualitative agreement with the exception of samples near the cutoff, which is to be expected for qualitative tests.

## INTERFERENCE FACTORS

The BTA test was analyzed by testing urine containing the potential interferants listed below. These potential interferants did not show interference in the BTA test at the levels indicated.

**Possible Urine Constituents**

| | *Highest Level Tested With No Interference* | *Level At Which Substance Interfered* |
|---|---|---|
| Hemoglobin | 100 mg/dl | No interference at maximum level tested (MLT) |
| White Blood Cells | $5 \times 10^3$ cells/ml | $5 \times 10^4$ cells/ml |
| Red Blood Cells | $1 \times 10^6$ cell/ml | No interference at MLT |
| Albumin | 1 g/dl | No interference at MLT |
| Bilirubin (unconjugated) | 2 mg/dl | No interference at MLT |
| IgG | 3.13 mg/dl | 6.25 mg/dl |
| Uric Acid | 250 mg/dl | No interference at MLT |
| Ascorbic Acid | 2.5 g/dl | 5.0 g/dl* |
| Ampicillin | 600 mg/dl | No interference at MLT |

**Therapeutic Agents**

| | *Highest Level Tested With No Interference* | *Level At Which Substance Interfered* |
|---|---|---|
| Doxorubicin-HCl | 10 mg/dl | No interference at MLT |
| Mitomycin | 10 mg/dl | No interference at MLT |
| Nitrofurantoin | 50 mg/dl | No interference at MLT |
| Phenazopyridine-HCl | 100 mg/dl | 200 mg/dl‡ |
| Thiotepa | 10 mg/dl | No interference at MLT |
| Trimethoprim | 50 mg/dl | No interference at MLT |
| Bacillus Calmette Guerin | 20 mg/dl | No interference at MLT |
| Finasteride | 2.5 mg/dl | No interference at MLT |
| Flutamide | 100 mg/dl | No interference at MLT |

* This is 25 times the peak mean level of Vitamin C if consumed at a level of 1 g/day.

‡ Two times peak mean level expected in urine

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